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Transcriptional mutagenesis-based reporter system for the analysis of DNA repair

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Motivation and Aim: Base excision repair (BER) is an important cellular process responsible for removing non-bulky DNA base modifications. The BER proficiency of human cells is commonly assessed either by analysis of cell extracts or using damaged plasmid DNA but these approaches have rather low sensitivity and specificity. As an alternative, the plasmids with defined positioned DNA lesions could be used [1].

Methods and Algorithms: The knockout of BER genes *APEX1*, *OGG1*, and *MUTYH* were generated using genome editing technology CRISPR/Cas9 from the parental 293FT cells. To detect the DNA repair in cells we use the assay based on plasmids containing single nucleotide substitution in the *EGFP* gene, which results in a loss of fluorescence [2]. The lesion is introduced in the position of the substitution. Inability to repair the DNA lesion in the transcribed strand leads to a heterogeneous mRNA pool with different ribonucleotides positioned opposite to the lesion ("transcriptional mutagenesis"). The evaluation of a cell's fluorescence by cell cytometry provides information on the level of aberrant transcripts and thus on the repair of the original lesion in DNA.

Results: Constructs contacting synthetic analogous of abasic sites were used to detect the repair in cells deficient in the main apurinic/apyrimidinic endonuclease APEX1. Despite the *APEX1* knockout, the repair efficiency of this lesion was considerable, which could indicate the presence of alternative repair pathways. We also examined the repair of 8-oxoguanine in *OGG1* and *MUTYH* knockout cells. 8-oxoguanine is efficiently repaired by OGG1 when paired with cytosine, and adenine is repaired by MUTYH when paired with 8-oxoguanine. Base pair 8-oxoguanine:adenine is non-canonical and arises during the replication.

Conclusion: The EGFP reporter system can efficiently be used to assess the repair of DNA base damages in different knockout cell lines. Besides, this system should allow discovery of alternative repair mechanisms.

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